Pathologic progression in amyotrophic lateral sclerosis (ALS) results from motor neuron death, while the clinical expression also reflects the compensatory effects of collateral reinnervation consequent to lower motor neuron loss. In a cross-sectional study of ALS subjects, we made comparisons between motor unit number estimation (MUNE) values and several measures reflecting collateral reinnervation, including isometric strength, compound muscle action potential (CMAP) amplitude, surface motor unit action potential (S-MUAP) amplitude, fiber density (FD), macro-EMG potential amplitude, turns-to-amplitude (T/A) ratio, and amplitude and recruitment pattern of low threshold voluntary motor units in elbow flexor muscles. Before comparisons were made, test-retest reproducibility of these measures was assessed in ALS subjects, and is highest for isometric strength, and lower but similar for EMG measures. When the effects of multiple comparisons are considered, borderline significant correlations are found between MUNE values and isometric strength. Neither MUNE values nor isometric strength are significantly correlated with macro-EMG amplitude, FD, T/A ratio, or amplitude and recruitment rate of low threshold voluntary motor units. There are significant correlations of CMAP and S-MUAP with MUNE values, but these are statistical artifacts with no independent interpretation. We conclude that collateral reinnervation prevents isometric strength and EMG measures from accurately reflecting lower motor neuron death in ALS. MUNE measurements are better suited to provide insight into the true natural history of the disease process and may be clinically useful to follow progression and response in drug trials. © 1993 John Wiley & Sons, Inc.

Key words: motor unit number estimation • EMG • strength • reproducibility • ALS
quantitative measurements of strength reveal linear rates of loss when measured during the progressive phase of the disease. From this relationship, isometric strength has been considered to be the best measure of motor unit activity in ALS. However, computer simulations modeling the compensatory effects of collateral reinnervation on motor unit loss fail to show linearity. Serial EMG measurements confirm nonlinear changes with disease progression. Accordingly, the true natural history of progression in ALS is to be distinguished from the clinical history of progression as determined by changes in strength and EMG measures.

An estimate of the number of surviving motor units in a muscle represents a direct measure of the disease state in ALS. Several electrophysiologic motor unit number estimation (MUNE) techniques have been used to study the rate of motor unit loss in ALS subjects, and preliminary data support nonlinear rates of loss.

A number of studies in ALS patients have compared muscle strength and EMG measures but few EMG measures have been evaluated simultaneously, and few studies include MUNE. We report the results of a cross-sectional study of ALS subjects comparing isometric strength and quantitative EMG measures, including MUNE. The purpose was to determine if measures of isometric strength, MUNE, and quantitative EMG are correlated with each other.

Before associations between these measures can be fully interpreted, it is necessary to have data on test–retest reproducibility of the measurements in ALS subjects. Reproducibility data for quantitative measurements of muscle strength are available for normal and ALS subjects, but data for quantitative EMG measurements are limited, and we therefore assessed the reliability of these measures in ALS subjects.

METHODS

Elbow flexor muscles were studied in 31 ALS subjects. All electrodiagnostic studies were performed on a Nicolet Viking EMG machine (Nicolet Instrument Corp., Madison, WI) using proprietary software. MUNE was performed in biceps-brachialis muscles by a modification of the spike-triggering averaging technique. A 7 × 80 mm silver strip stigmatic electrode was placed transversely across the motor point of the biceps-brachial muscle and a reference disc electrode was placed over the olecranon process. The maximal compound muscle action potential (CMAP) from the biceps-brachialis muscle group was obtained by percutaneous electrical stimulation of the musculocutaneous nerve. A single fiber macro-EMG recording electrode was introduced into the biceps muscle 3–5 cm distal to the stigmatic electrode and used to isolate the triggering motor unit spike potentials, which were generated by weak voluntary muscle contractions. The surface motor unit action potential (S-MUAP) from the triggered motor unit spike was recorded by the strip electrode. Spike potential stability during the recording epoch was monitored by a raster display of spike potentials. Each S-MUAP obtained for measurement was averaged approximately 200 times. Fifteen S-MUAPs were obtained from each muscle. The corresponding triggered motor unit spike potentials were recorded at five different depths and from three penetrations at medial, middle, and lateral sites across the muscle. The MUNE was calculated as the peak-to-peak CMAP amplitude divided by the mean peak-to-peak amplitude of 15 S-MUAPs. An S-MUAP was discarded only if it was identical in shape and amplitude to the preceding potential.

The mean and range of MUNE values obtained by the spike-triggered method in ALS subjects in this study, as well as the range and lower limiting values obtained in our laboratory from normal subjects, are similar to those reported by Brown and coworkers from the same muscle.

Simultaneously with recording the S-MUAP, the corresponding macro-EMG potential was recorded and averaged. The peak-to-peak amplitude was determined for each of 15 macro-EMG potentials, and the median value calculated. A macro-EMG potential was excluded if both it and the simultaneously recorded S-MUAP were identical in shape and amplitude to the preceding macro-EMG and S-MUAP waveforms.

Measurement of FD was made using the single fiber port of the macro-EMG electrode. Fiber density values were recorded from the three sites within the muscle, but the FD values were obtained separately from the macro-EMG potentials. Twenty values were obtained and averaged.

Quantitative measurement of the interference pattern was made by analysis of the turns-to-amplitude (T/A) ratio. A concentric needle EMG electrode was introduced into the biceps muscle at two penetration sites, and recordings made at different electrode depths and trajectories in the muscle. Various levels of voluntary muscle contraction, from weak to maximal, were recorded during 1-s epochs. Twenty recordings were made, and the measurement reported as the ratio of the num-
The number of turns per second divided by the average amplitude per turn.

The average amplitude of the first two or three motor unit action potentials (MUAPs) recruited during weak voluntary effort was estimated by visual inspection of the recruitment spike train, and expressed in millivolts. Motor unit recruitment was assessed at the same time and graded on a 0–4 scale, with 0 being normal and 4 maximally reduced recruitment. Isometric elbow flexion strength was measured on a Cybex II dynamometer and expressed as the mean of three values during the plateau phase at maximal effort.

Test–retest measurement reproducibility was assessed by: (1) the relative difference (calculated as the absolute value of the test–retest difference divided by their mean, and expressed as a percentage); and (2) the correlation between test and retest values. Test–retest differences were assessed for statistical significance by the Wilcoxon signed-rank test. Correlations between isometric strength, MUNE, or any of the EMG measures were assessed by (1) Pearson's r, and (2) stepwise multiple regression. Because multiple comparisons were made, attained significance levels of \( P < 0.02 \) were considered significant, levels of \( 0.02 < P < 0.05 \) marginally significant, and \( P > 0.05 \) not significant.

**RESULTS**

**Reproducibility of Test Measurements.** Test–retest reproducibility was investigated for isometric strength and quantitative EMG measures in ALS subjects. Testing and retesting was performed by the same tester, and usually within the same day (range 1–7 days, median 1.1 day). Reproducibility data are shown in Table 1, which includes values from MUNE reproducibility previously reported.\(^2\)

The mean test–retest relative difference was lowest for isometric strength (4.4%) and highest for MUNE (32.6%), while the differences for most of the EMG measures were between 15.9% and 27.9%. There were no significant differences between test and retest values for isometric strength, MUNE, or any of the EMG measures (\( P = 0.15 \) to 0.91). Test–retest correlation coefficients were lowest for MUNE (\( r = 0.54 \)); however, when ALS subjects with MUNE values less than 235 (the lower limit of the range in normal subjects)\(^2\) were considered separately, the correlation coefficient rose to \( r = 0.65 \). Correlation coefficients for isometric strength and the remaining EMG measures were between \( r = 0.81 \) and \( r = 0.99 \).

**Correlations between Test Measurements.** Correlations between MUNE values versus isometric strength and individual EMG measures were computed, with results in Table 2 and a scattergram of MUNE versus isometric strength in Figure 1. CMAP amplitude was most highly correlated with MUNE values (\( r = 0.61 \)), T/A ratio the least (\( r = -0.02 \)), and the remaining measures had intermediate coefficients (between \( r = 0.37 \) and \(-0.46 \)). Inspection of scattergrams of MUNE values plotted against isometric strength and EMG measures revealed three measures (S-MUAP amplitude, FD, and macro-EMG amplitude) that had outlying and potentially influential points, but there was little change in the correlations after removal of the points. When corrected for multiple comparisons, the only significant correlations were found between MUNE values and the EMG measures of CMAP amplitude and S-MUAP amplitude (Table 2). This was expected because both measures are used in the calculation of MUNE values. Measures of borderline statistical significance included FD,

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**Table 1.** Test–retest reproducibility of MUNE values and EMG measures in biceps-brachialis muscles, and isometric elbow flexion strength in ALS subjects.

<table>
<thead>
<tr>
<th>Measure</th>
<th>n</th>
<th>Relative difference (%)</th>
<th>Test–retest; Wilcoxon P-value</th>
<th>Correlation coefficient full (restricted*) data</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUNE</td>
<td>15</td>
<td>32.6</td>
<td>0.27</td>
<td>0.54 (0.65)</td>
</tr>
<tr>
<td>CMAP amplitude</td>
<td>15</td>
<td>21.4</td>
<td>0.91</td>
<td>0.85</td>
</tr>
<tr>
<td>S-MUAP amplitude</td>
<td>15</td>
<td>23.8</td>
<td>0.15</td>
<td>0.81</td>
</tr>
<tr>
<td>FD</td>
<td>12</td>
<td>15.9</td>
<td>0.21</td>
<td>0.83</td>
</tr>
<tr>
<td>Macro-EMG</td>
<td>13</td>
<td>27.9</td>
<td>0.25</td>
<td>0.98</td>
</tr>
<tr>
<td>Turns/amplitude ratio</td>
<td>9</td>
<td>19.7</td>
<td>0.77</td>
<td>0.91</td>
</tr>
<tr>
<td>Isometric strength</td>
<td>10</td>
<td>4.4</td>
<td>0.21</td>
<td>0.99</td>
</tr>
</tbody>
</table>

\(^{*}\) Correlation coefficient calculated for ALS subjects with MUNE values <235, the lower limit of the normal MUNE range (see text).

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**Table 2.** Correlations of MUNE values versus EMG measures in biceps-brachialis muscles and isometric elbow flexion strength in ALS subjects.

<table>
<thead>
<tr>
<th>Measure</th>
<th>n</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMAP amplitude</td>
<td>31</td>
<td>+0.61</td>
<td>0.0003</td>
</tr>
<tr>
<td>S-MUAP amplitude</td>
<td>31</td>
<td>-0.46</td>
<td>0.009</td>
</tr>
<tr>
<td>FD</td>
<td>31</td>
<td>-0.40</td>
<td>0.02</td>
</tr>
<tr>
<td>MUAP amplitude</td>
<td>31</td>
<td>-0.39</td>
<td>0.03</td>
</tr>
<tr>
<td>Isometric strength</td>
<td>31</td>
<td>+0.37</td>
<td>0.04</td>
</tr>
<tr>
<td>MUAP recruitment</td>
<td>31</td>
<td>-0.36</td>
<td>0.05</td>
</tr>
<tr>
<td>Macro-EMG</td>
<td>22</td>
<td>-0.29</td>
<td>0.20</td>
</tr>
<tr>
<td>Turns/amplitude ratio</td>
<td>28</td>
<td>-0.02</td>
<td>0.91</td>
</tr>
</tbody>
</table>
FIGURE 1. Scattergram of MUNE in biceps-brachialis muscles versus isometric elbow flexion strength in ALS subjects.

semiquantitative estimates MUAP amplitude and MUAP recruitment, and isometric strength (Table 2). Stepwise multiple regression analysis confirmed the strong association of CMAP and S-MUAP amplitudes with MUNE, and showed that once they were in the regression equation, the other EMG measures and isometric strength were not significantly associated with MUNE (P > 0.05).

ALS subjects with motor unit counts less than 235 had higher test–retest MUNE correlations (Table 1). Correlation coefficients were calculated separately for this subgroup, but coefficients were not substantially different from those calculated from the whole group.

Previous studies measured ALS progression by changes in isometric strength. Correlation of strength against MUNE and EMG measures are shown in Table 3. When corrected for multiple comparisons, no EMG measures were more highly correlated with strength (Table 3) than with MUNE values (Table 2). Isometric strength and MUNE values (Fig. 1) were the only independent measures reaching even borderline statistical significance (P = 0.04).

DISCUSSION

The first component of this study is an investigation of intrasubject test–retest reproducibility for isometric strength and quantitative EMG measures in ALS subjects. The results show that reproducibility is very high for isometric strength and lower for quantitative EMG measures. Among the EMG measures, reproducibility is somewhat less for MUNE, but all measures have relative differences between 15.9% and 32.6% (Table 1). MUNE is a ratio, and subject to the variability of both CMAP and S-MUAP measurements. When test–retest variability is assessed by correlation, coefficients for MUNE are influenced by outlying points. All ALS subjects had low MUNE values, and it is in this range that MUNE is most clinically useful.

Among the ALS subjects with test values below the normal lower limit (235), the coefficient rose to r = 0.65 (Table 1), and rose further to r = 0.91 when only more severely affected subjects with values less than 160 were considered.

There are few studies of reproducibility for comparison. Isometric elbow flexion strength measurements in ALS patients are highly reproducible, with reported intrasubject variability of 5.5–8.9% and correlation coefficients of r = 0.99, and our values are in good agreement. Test–retest variability of CMAP amplitude measured from anterior tibialis muscle in ALS subjects is reported to be 4% with a correlation coefficient of r = 0.97. In the current study, the greater difficulty in stimulating a proximal motor nerve (musculocutaneous nerve) may contribute to variability of 21.4%, although the correlation (r = 0.85) is quite high.

In ALS subjects, individual FD values are frequently very large, and single fiber potentials may display marked jitter and blocking. This makes accurate spike component counting unreliable, and FD calculations reflect minimum values under these conditions. Test–retest SD variability from anterior tibialis muscle in ALS subjects is reported to be 11.6% with correlation coefficient of r = 0.38, and our values are comparable.

Test–retest variability results of the macro-EMG potential amplitude are not available. Bilateral macro-EMG amplitude comparisons have been made in normal subjects and, if a measure of

| Table 3. Correlations of isometric elbow flexion strength versus EMG measures in biceps-brachialis muscles in ALS subjects. |
|-----------------|--------|----|---|
|                 | n     | r   | P-value |
| MUNE            | 31    | +0.37 | 0.04 |
| MUAP recruitment| 31    | −0.30 | 0.10 |
| CMAP amplitude  | 31    | +0.29 | 0.11 |
| Macro-EMG amplitude | 22 | −0.35 | 0.11 |
| FD              | 31    | −0.21 | 0.28 |
| S-MUAP amplitude| 31    | −0.17 | 0.36 |
| MUAP amplitude  | 31    | −0.17 | 0.38 |
| Turns/amplitude ratio | 28 | +0.08 | 0.67 |
variability can be inferred, the smaller macro-EMG values are within 10–30% of the larger values.\textsuperscript{35} Despite the greater variability of macro-EMG potential amplitudes in ALS subjects compared to normal subjects,\textsuperscript{32} our test–retest variability is comparable (Table 1).

Test–retest variability of the spike-triggered averaging method of MUNE has been reported and discussed by us, and the variability is similar to that described for other MUNE techniques.\textsuperscript{2}

The second component of this study is a comparison of clinical measures of isometric strength and quantitative EMG measures in a cross-section of ALS subjects. Multiple regression analysis shows that MUNE values are significantly correlated only with the measures of CMAP and S-MUAP amplitude, which are the two factors used to calculate the MUNE. Isometric strength is not significantly correlated with MUNE (Fig. 1) or any of the EMG measures. There are several reasons for the lack of significant correlations in ALS subjects. Changes in the size and intramuscular organization of motor units due to collateral reinnervation\textsuperscript{32} are not linearly related to the rate of motor neuron loss.\textsuperscript{18}

Further, strength and EMG measurements in this and other studies are made from different portions of the lower motor unit. Isometric strength is usually measured from a synergistic group of muscles, while routine EMG measures are made in a single muscle. MUNE may be made from a single or group of muscles. In addition, the various EMG electrodes record from different segments of the motor unit within muscle. Measurements of strength also include the effects of upper motor neuron loss, although signs of loss, such as spasticity, affect function, such as ambulation, more than isometric strength.\textsuperscript{17,25} Strength and T/A ratio measurements are also influenced by fatigue. Accordingly, poor correlations between isometric strength, MUNE values, and the other EMG measures are not unexpected.

Before making comparisons with other studies, several points should be emphasized. This is a cross-sectional study of correlations between measures, which is in contrast to longitudinal studies with correlations between single measures over time. With any progressive and irreversible disorder, all measures will eventually show change in the direction of deterioration, and when large samples of subjects are studied significant linear correlations with time are to be expected. What is more informative are relationships or differences between measures at a given stage of the disorder. We assume that the sample of ALS subjects represents different degrees of denervation, as reflected in the range of MUNE values which overlap with the normal range.\textsuperscript{2}

In longitudinal studies, isometric strength falls linearly when values from ALS subjects are combined, but values from individual subjects may show marked variability over time.\textsuperscript{17,26} In a similar fashion, average CMAP amplitude declines linearly with time when all ALS subjects are combined, but individual subjects may show poor correlations.\textsuperscript{17} In ALS subjects, FD rises early in the disease and may fall later.\textsuperscript{37} In serial studies, macro-EMG amplitude may rise, fall, or remain unchanged with time.\textsuperscript{32} These findings support nonuniform changes due to collateral reinnervation during ALS progression.

In cross-sectional studies, collateral reinnervation can compensate for mild-to-moderate motor unit loss with preservation of strength and CMAP amplitude.\textsuperscript{16,22} Reinnervation causes FD and macro-EMG amplitude to rise, but not in proportion to each other.\textsuperscript{32,37,38}

There are few studies which include comparisons between MUNE and other measures. In cross-sectional studies of single muscles in ALS subjects, poor correlations are found between MUNE values, twitch tension, macro-EMG amplitude, and CMAP amplitude.\textsuperscript{9,16,22} Correlation studies performed in subjects with other neuromuscular diseases serve to emphasize the dynamic features of collateral reinnervation. In a cross-sectional study of the vastus lateralis muscle in subjects with old poliomyelitis, there are no correlations between macro-EMG amplitude and isometric strength or FD.\textsuperscript{10} In a cross-sectional study of subjects with several types of dystrophy, no correlations are noted between FD and averaged S-MUAP amplitude.\textsuperscript{30} This is to be contrasted to findings in normal subjects (who have only age-related collateral reinnervation) where there is a reasonable correlation between averaged S-MUAP and macro-EMG amplitude.\textsuperscript{31}

Impaired motor unit recruitment should be a very sensitive measure of motor neuron loss,\textsuperscript{6} and recruitment receives greatest weight among EMG measures in a proposed set of criteria from the World Federation of Neurology for the diagnosis of ALS. Reduced recruitment of the first few motor units can be studied quantitatively by measuring motor unit discharge frequencies,\textsuperscript{28} but is usually estimated qualitatively by subjective assessment of firing frequency and the number of discharging
units. A commonly used five-point qualitative scale provides ordinal data only, and is more sensitive to mild than to severe reductions in recruitment. Accordingly, routine qualitative measures of recruitment are not expected to be significantly correlated with MUNE values or isometric strength.

Quantitative measurements of the interference pattern by T/A ratio analysis might be expected to be correlated with MUNE values. Several testing methods have been proposed with respect to the level of muscle contraction at which T/A ratio data are gathered. In this study, data were gathered at all levels of contraction. At high levels of effort, fatigue was common and prominent despite good subject cooperation. This was noted as a drop out of initially recruited motor units and a decline in force before the 1-s recording epoch could be completed. Fatigue is prominent in weaker subjects in whom higher levels of effort were required to generate even a moderately full interference pattern. Rest periods restored full activity, but fatigue returned with subsequent efforts. This drop out may represent failure at newly formed neuromuscular junctions or at axon branch points. There was evidence in most subjects for the former, with marked jitter and blocking on single fiber recording, but neurogenic blocking was not observed. The effects of fatigue on the T/A ratio in ALS subjects may be similar to that found in myasthenia gravis subjects in whom both increases and decreases of the ratio are observed. Despite a prominent component of fatigue, test–retest reproducibility of the T/A ratio does not differ in magnitude from other EMG measures. Under these conditions, there is no significant correlation between maximal isometric strength and T/A ratio values in our ALS subjects. It is possible that stronger correlations could occur if T/A ratios were determined at 30% of maximal force.

Changes in motor unit configuration are a marker for ALS, and quantitative measurements of MUAP duration and amplitude are sensitive measures of denervation. Late in the course of ALS, when motor units undergo decompensation, MUAP amplitude may fall and duration shorten. Accordingly, correlations of MUAP configuration with MUNE values or isometric force are not expected.

Previous studies of change in isometric strength and EMG measures have provided insight into the compensatory effects of collateral reinnervation in ALS. There are no significant correlations among these measures, which emphasizes underlying differences between the pathologic processes of motor neuron death and collateral reinnervation. MUNE is the only measure of lower motor neuron loss in this disorder, and there are several MUNE techniques available which can be applied to proximal and distal muscles. Reproducibility among the various techniques is similar, and will improve as online computer analysis is incorporated, permitting the averaging of multiple determinations. MUNE has been used to chart the rate of lower motor neuron loss in ALS subjects, and serial studies have prognostic value. MUNE has other clinical utilities. For example, combined data from MUNE and other EMG studies could provide information on whether a new therapeutic drug reduces the rate of lower motor neuron loss or increases collateral sprouting.

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